

**Effects of Long Term Exposure of 900-1800 MHz Radiation Emitted from 2G Mobile Phone on Mice Hippocampus-
A Histomorphometric Study** Narayanaperumal Mugunthan¹, Kathirvelu Shanmugasamy², Jayaram Anbalagan³,
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Abstract

Introduction

The advancement in the telecommunications technology with multi-functional added features in mobile phone, attracts more users of all age group. It is alarming to note that, the mobile phone use has increased amongst children and they are exposed to potentially harmful radiofrequency radiation in their lifetime.

Aim

To investigate the long term exposure of 900 to 1800 MHz radiations emitted from 2G mobile phone in mice hippocampus at histomorphometric level.

Materials and Methods

With due approval from institutional animal ethics committee, 36 mice were exposed to 2G mobile phone radiation, 48 minutes per day for a period of 30-180 days. The control group was kept under similar conditions without 2G exposure.

Mice were sacrificed and the brain was removed from the first month to six months period. Brain was removed from the cranial cavity and hippocampus region was disdivided out carefully and processed for routine histological study. Random serial divtions were analysed under microscope for histomorphometric changes. For statistical analysis, independent t-test was used for comparing control and 2G exposed groups.

Results

The mean density of neurons in the hippocampus regions CA1, CA2 and DGDB from first to sixth month was significantly lower in the 2G exposed groups; however, in CA3 and DGVB, the 2G exposed mice showed significantly higher density of neurons. The mean nuclear diameter of neurons in the hippocampus region of CA1, CA2, CA3, DGDB and DGVB from first to sixth months showed lower nuclear diameter in 2G exposed mice.

Conclusion

The long term exposure to 900-1800 MHz frequency radiations emitted from 2G mobile phone could cause significantly reduced neuron density and decreased nuclear diameter in the hippocampus neurons of mice.

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Introduction

Rapid developments in telephone technology made the communication faster and easier. The proliferation and the introduction of new generation mobile phones have created a widespread concern about public safety. Individuals of all ages, young and old use mobile phones. The amount of time for which one uses a mobile phone has also increased beyond limits.

Mobile phone is identified as a device emitting radiofrequency electromagnetic waves (RF-EMW). The second generation mobile phones (2G) operate on a frequency range of 900-1800 MHz [1]. Specific Absorption Rate (SAR) is the energy flow per unit of mass, i.e., watt per kilogram (W/kg). It is the measurement of power or heat absorbed by the body either in a local area of a human body tissue or averaged over the whole body. The International Commission on Non-Ionizing

Radiation Protection (ICNIRP) recommendations set a revised SAR limit of 1.6 W/Kg in 10 grams of tissue [2]. The Ministry of Commerce, India, has notified that imported handsets should bear self certification by manufacturers meeting the radiation standards. Even so, the absence of caution among mobile phone users regarding the levels of radiation discharged by handsets have turned into a noteworthy reason for worry on the wellbeing of these people. The Electromagnetic Fields (EMF) emitted by mobile phones has the potential to heat up human tissue in the head locale where the telephone is squeezed against. This thermal effect could cause heat on the ear, fatigue, headache, decreased concentration, dizziness, memory loss, tingling, numbness [3] and other severe potential long term damages such as genotoxicity [4]. We had conducted a study on the effects of chronic exposure to 2G and 3G cell phone radiations in mice testis and we found that radiation emitted from 2G and 3G cell phone caused reduction of serum testosterone level, microscopic changes in the seminiferous epithelium and reduction in the number of Sertoli and Leydig cells [5,6]. The hippocampus is involved in emotional behavior related to pain, declarative memory and stress. Neurons of hippocampus also exhibit plasticity. Plasticity represents the capacity of neurons to change in response to various stimuli or injury. Neuronal cells are related to synaptic activity as well as anatomical changes. Studies have shown that, chronic prenatal exposure to 900 MHz EMF caused pyramidal cell loss in the hippocampus of new-born rats [7]. Exposure to 2.4 W/kg GSM 1800 MHz microwaves during the early developmental stage could affect dendritic development and the formation of excitatory synapses of hippocampus neurons in culture [8]. Late studies reported that, no huge distinction in the neuronal populace of pyramidal cells in the cornu ammonis of the hippocampus [9] and sound-related pathways not influenced by long haul utilization of cellular telephone [10]. Yet another study reported that decreased memory functions in rats after long term exposure to GSM 900 mobile phone radiation [11].

The opposing exploratory reports on the impacts of cell telephone radiation on organic tissues raised much worry on human wellbeing. The rapid proliferation of wireless communication and expanded use on the other side has prompted us to undertake the present study, to assess the possible impacts of chronic exposure of 900-1800 MHz radiations transmitted from 2G cell phone on the hippocampus of mice model.

This study has been designed to evaluate the damage if any, caused by radiofrequency radiation emitted from 2G mobile phones on the hippocampus of mice for a period of 0-6 months.

Aim

To investigate the structural changes in quantitative terms utilizing stereological and histomorphometric information to prove or disprove the potential harm brought on by Radio Frequency Radiation (RFR) exposure with non-exposure group.

Materials and Methods

This study was approved by the Institutional Animal Ethics Committee and the study was conducted from August 2011 to October 2015 at Mahatma Gandhi Medical College and Research Institute, Puducherry, India. Seventy two neonatal albino mice procured from the King Institute of Preventive Medicine and Research, Animal section, Chennai, constituted the materials for the present study.

New born mice were randomly divided into 2 independent groups; control and 2G mobile phone radiation exposed group. All the mice were kept in cages in the central animal house at $22\pm 1^{\circ}\text{C}$, 60% relative humidity, adequate ventilation and 12 hours of illumination alternated with 12 hours of darkness. During the study, the mice were fed with laboratory diet and water ad libitum. Thirty six mice were exposed to 900-1800 MHz frequency radiation emitted from 2G mobile phone and thirty six mice were the control group without exposure from 2G mobile phone.

The roof of the mice cage was designed to hang the mobile phone from a distance of five cm from the floor. This distance allowed the mice to move freely and to avoid direct thermal injury. A 2G mobile phone was kept in non-vibrating, silent, Do Not Disturb (DND) with auto answer activated mode, inside the cage [5]. A 2G mobile phone operates on a frequency bandwidth of 900-1800 MHz and with the power of 1.6 W/Kg. The highest SAR value for this standard handset is 1.69 W/Kg (10gm of tissue) and this SAR value is inside the breaking point of the International Commission on Non-Ionizing Radiation Protection (ICNIRP) recommendation [6].

The 2G phone kept inside the cage was rung upon from another 2G phone. Every call continued for two minutes, at every 30 minutes period. The animals were exposed to 2G radiation for 48 minutes per day for a 12 hour period. The total duration of exposure was from 30-180 days. Radiofrequency meter was used to measure the amount of radiation exposed in the experimental mice. The 36 mice in control group were kept under similar conditions but without exposure to 2G cell phone radiation. Six mice were sacrificed toward the end of 30, 60, 90, 120, 150 and 180 days of exposures in the experimental groups following 24 hours of last exposure [6]. An equal number of control mice were sacrificed on similar experimental design.

The brain was removed from the cranial cavity by careful dissection. The hippocampus region was identified and dissected out from the rest of the brain. The hippocampus region was fixed in 4% formalin solutions for 24 hours and was processed by routine histological procedures. The hippocampus region was sectioned at eight microns thickness and was stained with Haematoxylin & Eosin, toluidine blue and silver nitrate stains. The stained sections were analysed from random slide, random sections and random field using Olympus Trinocular Microscope (Model CX41, Japan). For capturing images Olympus E420 Camera mounted on Trinocular microscope was utilized.

In the hippocampus region of the brain, stratum pyramidalis of CA1, CA2, CA3 and stratum granular of Dentate Gyrus Dorsal Blade/superior limb (DGDB) and Ventral Blade/Inferior Limb (DGVb) were examined [Table/Fig-1]. (Adopted from

Susan Standring, Gray's Anatomy. The anatomical basis of clinical practice, 41st Edition, 2016. Elsevier publication).
[Table/Fig-1]:

Schematic diagram showing hippocampus and dentate gyrus.(Adopted from Susan Standring, Gray's Anatomy. The anatomical basis of clinical practice. 41st Edition, 2016. Elsevier publication)

a) Calculation of Neuronal Density

Neuronal density of CA1, CA2, CA3, and DGDB and DGVB was estimated using square reticule mounted on an eyepiece. The reticule was superimposed over the hippocampus region under 100x and randomly selected 20 small square areas were used for counting the neuron [Table/Fig-2].

[Table/Fig-2]:

Reticule mounted on an eyepiece to estimate neuronal density in the hippocampus.

b) Measurement of Neuronal Nuclear Diameter

Neuronal nuclear diameter of CA1, CA2, CA3, and DGDB and DGVB were measured using calibrated ocular micrometer under 100x [Table/Fig-3].

[Table/Fig-3]:

Calibrated ocular micrometer mounted on an eyepiece to measure neuronal nuclear diameter in the hippocampus.

Statistical Analysis

Independent t-test was used for comparing control and 2G exposed group. The p-value ≤ 0.05 is considered statistically significant.

Results

Microscopic Features of Hippocampus

Sections of hippocampus region from the first to the sixth month of control and 2G radiation exposed groups were scanned under a light microscope. The cells of CA1, CA2, CA3, DGDB and DGVB regions were studied under 100x, 400x and 1000x magnifications.

Control Group

The hippocampus proprius exhibited 3 layers: stratum oriens, stratum pyramidale, and a molecular zone. The CA3 region started from the mouth of the dentate gyrus region and ended at the junction between CA3 and CA2 region. The CA2 region was located between CA3 and CA1. The junction between CA2 and CA1 was marked by change of large pyramidal cells in the CA2 region to small tightly packed pyramidal cells in the CA1 region. The stratum pyramidale of CA1 showed 3-4 rows of small pyramidal neurons, with deeply stained round nuclei with 2-3 prominent nucleoli. In CA2, pyramidal neurons were larger in size and densely packed. Pyramidal neurons showed basophilic stained round to oval nuclei with prominent nucleoli. Many glial cells were seen among the pyramidal neuron with dark basophilic stained small rounded nuclei. The CA3 region showed mostly large pyramidal neuron and was loosely packed. Fine nerve fibers were also seen around the large pyramidal neurons in this region. The interstitium appeared normal with blood vessels. Dentate gyrus appeared with three layers: the molecular, the granular and the polymorphic cell layer. The stratum granulosum of DGVB (Dentate Gyrus Ventral Blade) and DGDB (Dentate Gyrus Dorsal Blade) showed densely packed small rounded granular neurons. The nucleus was rounded and stained dark basophilic. Few astrocytes and microglial cells were also seen. The subgranular zone of the dentate gyrus showed oval shaped pyramidal neurons with darkly stained nuclei [Table/Fig-4, 1a, 2a, 3a, 4a & 5a].

[Table/Fig-4]:

Photomicrograph showing hippocampus of control & 2G mice. A – Control & B -2G exposed hippocampus, under 40X. 1A, 2A, 3A, 4A, 5A, 6A, 7A & 8A- control group, 1B, 2B, 3B, 4B, 6B, 7B & 8B -2G exposed group, under 1000X. PN - Pyramidal neurons, GN - Granular neurons, * - Wide interstitium, Arrow – nerve fibers. A-5B (H & E stain), 6A-7B (Toluidine blue stain), 8A & 8B –Silver nitrate stain.

Under toluidine blue stain, neurons of CA1, CA2, CA3, DGDB and DGVB regions showed chromatin and nucleoli within the nucleus. Acidophilic cytoplasm appeared as pink stained structure encompassing the nucleus because of the positive charges on arginine and lysine [Table/Fig-4, 6a & 7a]. Under silver nitrate stain, the neurons and myelinated nerve fibers in the regions of CA1, CA2, CA3, DGDB and DGVB appeared black and normal [Table/Fig-4, 8a].

2G Exposed Group

The histological features were similar to the control group except, the pyramidal neurons in the CA1 and CA2 that were less densely packed. Similarly, granular neurons in the DGDB region were less densely packed. In contrast to the above, the pyramidal neurons in the CA3 region and, DGVB region were densely packed. The nucleus of the pyramidal neuron in the regions of CA1, CA2, CA3 and nucleus of granular neuron in the regions of DGDB & DGVB appeared smaller in comparison to the control group. The interstitium appeared wider in the region of CA1 and CA2 region, especially in the first and second months. Nerve fibers and the myelination appeared normal in the hippocampus proper and dentate gyrus region [Table/Fig-4, 1b, 2b, 3b, 4, 5b, 6b, 7b & 8b].

Histomorphometric Study

a) Mean Density of Neurons in the Hippocampus Region

A comparison between control and 2G exposed groups by independent t-test showed the mean density of neurons in the hippocampus regions CA1, CA2 and DGDB from first to sixth month were significantly lower in the 2G exposed group (p-value < 0.001); however, in CA3 (except in 2nd month) and DGVB the 2G exposed mice showed significantly higher density of neurons than the control group (p-value < 0.001) [Table/Fig-5].

[Table/Fig-5]:

Comparison of control and 2G exposed mice in terms of hippocampal neuron density of CA1, CA2, CA3, DGDB & DGVB.

Month	Control mice					2G exposed mice						Comparison by independent t-test (p-value)
	Mean neuron density/unit area of 0.0625 cm ²	S.D	S.E	95% C.I for Mean		Mean neuron density/unit area of 0.0625 cm ²	S.D	S.E	95% C.I for Mean			
				U.L	L.L				U.L	L.L		
CA1												
1	3.36	1.08	0.15	3.05	3.67	2.62	0.95	0.13	2.35	2.89	0.001*	
2	4.70	0.7	0.1	4.5	4.90	2.62	0.7	0.1	2.4	2.83	0.001*	

		1	0	0			5	1	1		
3	4.16	0.7 4	0.1 0	3.9 5	4.37	2.36	0.6 3	0.0 9	2.1 8	2.54	0.001*
4	4.38	0.5 3	0.0 8	4.2 3	4.53	2.30	0.5 4	0.0 8	2.1 5	2.45	0.001*
5	4.36	0.4 9	0.0 7	4.2 2	4.50	2.32	0.4 7	0.0 7	2.1 9	2.45	0.001*
6	4.92	0.9 0	0.1 3	4.6 6	5.18	2.94	0.7 1	0.1 0	2.7 4	3.14	0.001*
CA2											
1	2.36	0.7 8	0.1 1	2.1 4	2.58	1.74	0.5 7	0.0 8	1.5 8	1.90	0.001*
2	3.94	0.8 2	0.1 2	3.7 1	4.17	2.54	0.6 1	0.0 9	2.3 7	2.71	0.001*
3	2.80	0.9 0	0.1 3	2.5 4	3.06	1.34	0.4 8	0.0 7	1.2 0	1.48	0.001*
4	2.88	0.7 2	0.1 0	2.6 8	3.08	1.32	0.4 7	0.0 7	1.1 9	1.45	0.001*
5	2.84	0.8 2	0.1 2	2.6 1	3.07	1.72	0.5 7	0.0 8	1.5 6	1.88	0.001*
6	3.10	0.6 8	0.1 0	2.9 1	3.29	1.62	0.5 7	0.0 8	1.4 6	1.78	0.001*
CA3											
1	1.32	0.4 7	0.0 7	1.1 9	1.45	3.68	0.4 7	0.0 7	3.5 5	3.81	0.001*
2	5.66	0.9 4	0.1 3	5.3 9	5.93	5.90	1.1 3	0.1 6	5.5 8	6.22	0.251
3	1.68	0.4 7	0.0 7	1.5 5	1.81	3.20	0.9 7	0.1 4	2.9 2	3.48	0.001*
4	1.70	0.4	0.0	1.5	1.83	3.58	0.9	0.1	3.3	3.84	0.001*

		6	7	7			1	3	2		
5	1.72	0.5 0	0.0 7	1.5 8	1.86	3.52	1.0 2	0.1 4	3.2 3	3.81	0.001*
6	1.68	0.5 1	0.0 7	1.5 3	1.83	3.70	0.5 1	0.0 7	3.5 6	3.84	0.001*
DGDB											
1	4.34	0.4 8	0.0 7	4.2 0	4.48	2.56	0.9 3	0.1 3	2.3 0	2.82	0.001*
2	5.26	0.5 7	0.0 8	5.1 0	5.42	3.52	1.3 0	0.1 8	3.1 5	3.89	0.001*
3	5.08	1.0 1	0.1 4	4.7 9	5.37	3.36	0.7 5	0.1 1	3.1 5	3.57	0.001*
4	6.34	1.2 7	0.1 8	5.9 8	6.70	3.58	1.1 3	0.1 6	3.2 6	3.90	0.001*
5	5.64	0.4 9	0.0 7	5.5 0	5.78	3.14	0.8 6	0.1 2	2.9 0	3.38	0.001*
6	6.12	1.1 5	0.1 6	5.7 9	6.45	3.80	1.0 1	0.1 4	3.5 1	4.09	0.001*
DGVB											
1	3.38	0.5 3	0.0 8	3.2 3	3.53	6.68	1.2 4	0.1 8	6.3 3	7.03	0.001*
2	6.74	1.0 5	0.1 5	6.4 4	7.04	7.92	0.9 2	0.1 3	7.6 6	8.18	0.001*
3	5.40	0.8 8	0.1 3	5.1 5	5.65	7.10	1.2 8	0.1 8	6.7 4	7.46	0.001*
4	6.70	1.4 7	0.2 1	6.2 8	7.12	8.32	1.1 5	0.1 6	7.9 9	8.65	0.001*
5	6.64	1.3 7	0.1 9	6.2 5	7.03	8.26	1.1 0	0.1 6	7.9 5	8.57	0.001*
6	6.50	1.3	0.1	6.1	6.87	8.46	0.9	0.1	8.1	8.74	0.001*

		1	9	3			9	4	8		
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n = 50 in each group, * P-value statistically significant (≤ 0.05), CA-CornuAmmonis, S.D – Standard Deviation, S.E – Standard Error, CI – Confidence Interval, L.L – Lower Limit, U.L – Upper Limit.

b) Mean Nuclear Diameter of Neurons in the Hippocampus Regions

A comparison between control and 2G exposed groups by independent t-test was done. The mean nuclear diameter of neurons in the hippocampus region of CA1, CA2, CA3, DGDB and DGVB from first to sixth months (except CA3 region in 2nd month, DGDB region in 4th and 6th month and DGVB region in 3rd month) showed a lower nuclear diameter in 2G exposed mice than in control mice (p-value < 0.001) [Table/Fig-6].

[Table/Fig-6]:

Comparison of control and 2G exposed mice in terms of hippocampal neuronal nuclear diameter of CA1, CA2, CA3, DGDB & DGVB.

Month	Control mice					2G exposed mice					Comparison by independent t test(p-value)
	Mean nuclear diameter(μ)	S.D	S.E	95% C.I for Mean		Mean nuclear diameter(μ)	S.D	S.E	95% C.I for Mean		
				U.L	L.L				U.L	L.L	
CA1											
1	7.78	0.78	0.11	7.56	8.00	5.15	0.91	0.13	4.89	5.41	0.001*
2	6.29	0.60	0.09	6.12	6.46	5.14	0.66	0.09	4.95	5.33	0.001*
3	5.82	0.56	0.08	5.66	5.98	4.58	0.48	0.07	4.45	4.72	0.001*
4	6.64	1.25	0.18	6.29	7.00	5.70	0.94	0.13	5.44	5.97	0.001*
5	7.02	1.22	0.17	6.67	7.37	5.40	0.59	0.08	5.24	5.57	0.001*
6	6.84	0.68	0.10	6.64	7.03	5.37	0.80	0.11	5.14	5.60	0.001*

CA2											
1	9.88	0.7 7	0.1 1	9.66	10.10	5.51	0.8 8	0.1 2	5.26	5.76	0.001*
2	7.81	1.0 2	0.1 4	7.52	8.10	5.19	0.7 2	0.1 0	4.98	5.39	0.001*
3	8.40	0.9 1	0.1 3	8.14	8.66	5.63	0.6 7	0.1 0	5.44	5.82	0.001*
4	8.40	0.8 6	0.1 2	8.16	8.65	5.71	0.8 6	0.1 2	5.46	5.95	0.001*
5	8.99	0.8 2	0.1 2	8.75	9.22	5.90	0.9 0	0.1 3	5.65	6.16	0.001*
6	9.23	0.6 7	0.1 0	9.04	9.43	6.29	1.3 2	0.1 9	5.91	6.66	0.001*
CA3											
1	7.70	0.9 8	0.1 4	7.42	7.98	6.28	1.1 8	0.1 7	5.94	6.61	0.001*
2	5.61	0.6 9	0.1 0	5.42	5.81	5.51	1.3 1	0.1 9	5.14	5.88	0.620
3	7.44	0.8 3	0.1 2	7.20	7.68	6.18	0.7 2	0.1 0	5.97	6.38	0.001*
4	7.24	0.7 1	0.1 0	7.04	7.45	6.02	0.9 0	0.1 3	5.76	6.27	0.001*
5	8.02	1.0 5	0.1 5	7.72	8.31	5.82	0.6 1	0.0 9	5.65	6.00	0.001*
6	7.32	0.8 4	0.1 2	7.09	7.56	5.95	1.4 6	0.2 1	5.54	6.37	0.001*
DGDB											
1	5.29	0.5 9	0.0 8	5.12	5.45	4.72	0.6 3	0.0 9	4.54	4.90	0.001*
2	5.46	0.6	0.0	5.28	5.63	4.85	0.8	0.1	4.62	5.08	0.001*

		1	9				1	1			
3	5.09	0.5 9	0.0 8	4.92	5.25	4.81	0.6 9	0.1 0	4.62	5.01	0.033*
4	4.79	0.5 3	0.0 8	4.64	4.94	4.94	0.6 9	0.1 0	4.74	5.14	0.221
5	4.81	0.5 8	0.0 8	4.64	4.97	4.58	0.5 2	0.0 7	4.44	4.73	0.045*
6	4.80	0.5 9	0.0 8	4.63	4.97	4.98	0.7 1	0.1 0	4.77	5.18	0.180
DGVb											
1	5.84	0.8 9	0.1 3	5.59	6.10	4.94	0.9 1	0.1 3	4.68	5.20	0.001*
2	5.06	0.4 9	0.0 7	4.93	5.20	4.64	0.6 3	0.0 9	4.46	4.82	0.001*
3	5.10	0.5 3	0.0 8	4.95	5.26	5.04	0.7 8	0.1 1	4.82	5.26	0.633
4	5.69	0.9 9	0.1 4	5.41	5.97	5.14	0.9 3	0.1 3	4.88	5.41	0.005*
5	5.40	0.4 9	0.0 7	5.27	5.54	4.75	0.7 4	0.1 1	4.54	4.96	0.001*
6	5.57	0.7 5	0.1 1	5.35	5.78	4.95	0.7 5	0.1 1	4.74	5.16	0.001*

n = 50 in each group, * P-value statistically significant (≤ 0.05), CA-CornuAmmonis, S.D – Standard Deviation, S.E – Standard Error, CI – Confidence Interval, L.L – Lower Limit, U.L – Upper Limit.

Discussion

In the present study, histological analysis showed the pyramidal neurons in the CA1, CA2, CA3 and granular neurons in

DGDB and DGVB region appeared normal in both groups. Robert E. Anderson opined that the brain could be severely disturbed by the exposure of small amount of radiations during the embryonic period. Mature nervous tissue showed resistant to acute morphologic changes but functional abnormalities were experienced following non-ionizing radiations [12]. This could be the cause for the 2G exposed neurons not exhibiting morphological changes in the present study. TimoKumlin et al., also observed no degenerative changes, dying neurons or effects on the brain barrier following long term exposure to 900 MHz radiofrequency radiation [13]. A similar study conducted by Ragbetli MC et al., on the prenatal mice exposed to 890-915 MHz mobile phone radiations, reported no significant changes in the development of the hippocampus [14].

In the present study, the interstitium appeared wide in the CA1 and CA2 region especially in the first and second months of the 2G group in comparison to the control group. The probable cause could be due to radiation induced endothelial damages, vasodilatation and increased vascular permeability resulting in the interstitial oedema.

a) Neuronal Density

In the present study, the mean density of neurons per unit area in the hippocampus region of 2G exposed groups indicated significantly lower in the regions of CA1, CA2 and DGDB than the control group. However, the region of CA3 and DGVB of the 2G group showed higher neuron density than the control group. The possible reason for the decreased neuronal density could be the reduction in the number of pyramidal/granular neurons or increased intercellular space due to oedema or due to the migration of neurons towards the stimulus of RFR. Bas O et al., and Odaci E et al., reported that, 900 MHz EMF caused a reduction in the number of pyramidal cells in the hippocampus region and granule cells in the dentate gyrus [15,16]. They also reported increased number of damaged cell density and dark cell. However, in the present study, damaged neurons are not significant.

The higher neuronal density in the CA3 and DGVB region of the 2G group could be due to the production and plasticity of new neurons or due to the migration of neurons towards the stimulus of RFR. Although, the production of dentate gyrus granule cells starts prenatally, the higher differentiation generally takes place in the first 20 days after birth [16].

Roberto Piacentini et al., reported that, exposure to extremely low-frequency EMF increases the expression and function of voltage-gated Ca^{2+} channels. The Ca^{2+} convergence through Cav1 channels assumes a key part in advancing neuronal differentiation of neural stem/progenitor cells [17]. Hiroki Toda et al., expressed that high-frequency stimulation to the anterior nucleus of thalamus expands the hippocampus neurogenesis and reestablishes tentatively stifled neurogenesis [18].

b) Neuronal Nuclear Diameter

The mean nuclear diameter of the 2G group neurons in CA1, CA2, CA3, DGDB and DGVB was significantly lower than the control except CA3 neurons in the 2nd month, DGDB neurons in the 4th and 6th month and DGVB neurons in the 3rd month. The nucleus of the neurons was more susceptible to radiation than the cytoplasm. At a low dose of radiation, the nuclear chromatin appeared somewhat clumped, swollen and oedematous. At moderate to high dose of radiation, the nucleus appeared pyknotic and showed karyorrhexis [12]. In the present study, the low dose radiation emitted from the 2G cell phone showed nuclear changes such as swollen and oedematous nucleus in the pyramidal neurons of CA3 neurons in the 2nd month, DGDB neurons in the 4th & 6th month and DGVB neurons in the 3rd month. However, absence of pyknotic nuclei or karyorrhexis was observed and this in accordance to the reports by Robert E Anderson.

Limitation

Whether the changes observed due to 2G mobile phone radiation exposure, are reversible or not on withdrawing the exposure, warrants further research.

The direct extrapolation of the outcome of present study to human population may be limited due to differences in species, volume and size, life span, functional and anatomical organization of brain, the duration of exposure, frequency and intensity of transmission, the shape and size of the exposed brain, the water and mineral content of the brain and also the distance from the radiation source.

Conclusion

We conclude that long term exposure to ultra-high frequency radiation emitted from a 2G mobile phone could cause reduction in the density of neurons in the hippocampus region of CA1, CA2 and DGDB; increased neuron density in CA3 and DGVB region. Similarly, significantly lower nuclear diameter in the hippocampus region of CA1, CA2, CA3, and DGDB & DGVB of 2G exposed neurons. Thus the changes observed in the hippocampus neurons could bring about signs and symptoms connected with headache, lack of concentration, memory, anxiety and emotion.

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